

AMENDMENTS TO THE SPECIFICATION

Please replace paragraph [0019] with the following:

[0019] Figure 3 is a line graph depicting the radiolabeling formation kinetics of ¹⁷⁷Lu DOTA-Herceptin™ ¹⁷⁷Lu DOTA-HERCEPTIN™ radioimmunoconjugates.

Please replace paragraph [0020] with the following:

[0020] Figure 4 is a line graph depicting the *in vitro* serum stability as measured by instant thin layer chromatography (ITLC) of ¹⁷⁷Lu DOTA-Herceptin™ ¹⁷⁷Lu DOTA-HERCEPTIN radioimmunoconjugates.

Please replace paragraph [0021] with the following:

[0021] Figure 5 is a line graph depicting the *in vitro* serum stability as measured by size exclusion high performance liquid chromatography (SE-HPLC) of ¹⁷⁷Lu DOTA-Herceptin™ ¹⁷⁷Lu DOTA-HERCEPTIN radioimmunoconjugates.

Please replace paragraph [0022] with the following:

[0022] Figure 6 is a bar graph illustrating the comparison of the uptake in bone of Herceptin™ HERCEPTIN immunoconjugates radiolabeled with ¹⁷⁷Lu.

Please replace paragraph [0115] with the following:

[0115] This example demonstrates the conjugation of Herceptin™ HERCEPTIN with C-DOTA, PA-DOTA, 1B4M-DOTA (7), and CHX-DOTA (14).

Please replace paragraph [0116] with the following:

[0116] The Herceptin™ HERCEPTIN was generously provided by Dr. R. Altemus (Radiation Oncology Branch, NCI). The Herceptin™ HERCEPTIN was concentrated to 5 mg/mL and conjugated with either 2-(*p*-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (C-DOTA), 1,4,7,10-tetraaza-N-(1-carboxy-3-(4-nitrophenyl)propyl)-N',N'',N'''-tris(acetic acid) cyclododecane (PA-DOTA), 1B4M-DOTA (7), or CHX-DOTA (14), employing the linkage methods for aryl isothiocyanato groups that have been well described in the literature (see, for example, Mirzadeh et al.,

Bioconjugate Chem. 1, 59-65 (1990)). Unreacted or "free" ligand was separated from the conjugated antibody by dialysis in 0.15 M NH₄OAc. The average number of chelates per antibody for the conjugation products was about 1 chelate per protein moiety, as determined by the appropriate spectrometric method for these chelating agents (Dadachova et al., *Nucl. Med. Biol.* 26, 977-982 (1999); Pippin et al., *Bioconjugate Chem.* 3, 342-345 (1992)). Protein concentration was determined using the Lowry method with a standard of bovine serum albumin (Lowry et al., *J. Biol. Chem.*, 193, 265-275 (1951)).

Please replace paragraph [0117] with the following:

[0117] This example demonstrates radiolabeling and comparative radiolabeling of the C-DOTA-, PA-DOTA-, 1B4M-DOTA (7)-, and ~~CHX-DOTA (14)-~~Hereceptin™ CHX-DOTA (14)-HERCEPTIN immunoconjugates prepared in Example 15.

Please replace paragraph [0118] with the following:

[0118] Radiolabeling with ¹⁷⁷Lu of the four immunoconjugates was performed analogously to previous reports (see, for example, Ruegg et al., *Cancer Res.* 50, 4221-4226 (1990)). The ¹⁷⁷Lu (1-3 mCi in 10-20 µL 0.1 M HCl) (U. Missouri, Columbia) was added to about 200 µL 0.15 M NH₄Ac buffer (pH 5.0-5.5) containing 300-400 µg of each of the ~~DOTA-Hereceptin™~~ DOTA-HERCEPTIN immunoconjugates prepared in Example 15. The reaction mixtures were incubated at 37 °C for 1-2.5 h. The reaction kinetics were followed by taking aliquots at different times and analyzing the components using ITLC developed in 10 mM EDTA/0.15 M NH₄OAc (Figure 3). The reactions were halted by adding 5 µL of 0.1 M DTPA. The reaction yields were determined by the ITLC method described previously (Ma et al., *Nucl. Med. Biol.*, 29, 91-105 (2002)). The ~~¹⁷⁷Lu-DOTA-Hereceptin™~~ ¹⁷⁷Lu-DOTA-HERCEPTIN conjugates were purified through a 10-DG desalting column (Bio-Rad, Hercules, CA) eluted using PBS and the antibody peaks were collected. Purity of the ¹⁷⁷Lu radiolabeled ~~DOTA-Hereceptin™~~ DOTA-HERCEPTIN radiolabeled immunoconjugates was determined using ITLC and/or size exclusion HPLC (SE-HPLC).

Please replace paragraph [0119] with the following:

[0119] Radio-iodination of Hereceptin™ HERCEPTIN with Na ¹²⁵I was performed as described using the Iodogen method (Fraker et al., *Biochem. Biophys. Res. Commun.*, 80,

849-857 (1978)). The product was purified using a desalting column (PD-10; Amersham Biosciences, Piscataway, NJ).

Please replace paragraph [0121] with the following:

[0121] The immunoreactivity of the RIC was assessed in a live-cell radioimmunoassay (RIA) as detailed elsewhere (Garmestani et al., *Nucl. Med. Biol.*, 29, 599-606 (2002)). HER2 positive cells (N87) were harvested, pelleted at 1,000 x g (Allegra 6KR; Beckman Coulter, Palo Alto, CA) and resuspended in PBS (pH 7.2) containing 1% BSA and were added to 12 x 75 mM polypropylene tubes (1 x 10⁶ cells in 100 µL). Serial dilutions of the radiolabeled ~~Hereceptin™~~ HERCEPTIN preparations (about 200,000 cpm -12,500 cpm in 50 µL) were then added in duplicate and gently shaken. Following an overnight incubation at 4 °C, the cells were washed once with 4 mL of 1% BSA in PBS and pelleted at 1,000 x g for 5 min, and the supernatant was decanted. The pelleted cells were then counted in a γ-scintillation counter (Packard), and the percent binding was calculated for each dilution. The values presented in Table 1 are an average of the serial dilutions. To confirm the specific reactivity of the RIC, cells were incubated with about 200,000 cpm of the RIC along with an excess (10 µg) of unlabeled ~~Hereceptin™~~ HERCEPTIN.

Please replace paragraph [0123] with the following:

[0123] An *in vitro* serum stability study was performed with all four of the above radioimmunoconjugates prepared in Example 16 with the measurements being determined by ITLC and size exclusion high performance liquid chromatography (SE-HPLC) methods over two months. The purified ~~¹⁷⁷Lu-DOTA-Hereceptin™~~ ¹⁷⁷Lu-DOTA-HERCEPTIN conjugates (2 mL each) were mixed with 2 mL human serum (Gemini Bioproducts, Woodland, CA). The mixtures were maintained in a 5% CO₂ incubator at 37 °C. At different time points, 50 µL aliquots were taken, mixed with 5 µL of 0.1 M DTPA, and incubated at 37 °C for 30 min. The percentage of ¹⁷⁷Lu associated with the immunoconjugate was analyzed by both ITLC (Figure 3) and SE-HPLC (Figure 4). The ~~¹⁷⁷Lu-DOTA-Hereceptin™~~ ¹⁷⁷Lu-DOTA-HERCEPTIN conjugate demonstrated superior stability among the four different DOTA immunoconjugates that were investigated. Both of the ¹⁷⁷Lu-C-DOTA and the ~~1B4M-DOTA~~ 1B4M-DOTA ~~(7)-Hereceptin™~~ (7)-HERCEPTIN conjugates exhibited similar stability. The

¹⁷⁷Lu-CHX-DOTA-(14)-Hereceptin™ ¹⁷⁷Lu-CHX-DOTA (14)-HERCEPTIN conjugate appeared to be less stable.

Please replace paragraph [0124] with the following:

[0124] This example demonstrates *in vitro* stability of a compound of formula [III] (PIP-DOTA) conjugated to Hereceptin™ HERCEPTIN and complexed to a radioisotope.

Please replace paragraph [0125] with the following:

[0125] An *in vitro* serum stability study was performed on piperidiny-substituted DOTA-Hereceptin™ DOTA-HERCEPTIN conjugates, as prepared in Example 15, that were complexed to ²⁰⁵Bi, ¹⁵³Gd or ⁸⁶Y, as prepared in Example 16. The purified PIP-DOTA radioimmunoconjugates (2 mL each) were mixed with 2 mL human serum (Gemini Bioproducts, Woodland, CA). The mixtures were maintained in a 5% CO₂ incubator at 37 °C. At different time points, 35 µL (²⁰⁵Bi) or 80 µL (¹⁵³Gd and ⁸⁶Y) aliquots were taken, mixed with 5 µL of 0.1 M DTPA, and incubated at 37 °C for 30 min. The percentage of ²⁰⁵Bi, ¹⁵³Gd or ⁸⁶Y associated with the PIP-DOTA immunoconjugate was analyzed by SE-HPLC. The ²⁰⁵Bi and ¹⁵³Gd conjugates demonstrated superior stability over time.

Please replace paragraph [0127] with the following:

[0127] The radioimmunoconjugates (RICs) were compared *in vivo* using athymic mice bearing human colon adenocarcinoma xenografts. Female athymic mice (nu/nu), obtained from Charles River Laboratories (Wilmington, MA) at 4-6 weeks of age, were injected subcutaneously on the flank with 2 x 10⁶ LS-174T cells in 0.2 mL of RPMI-1640. At approximately 10-14 days, when the tumors measured between 0.4-0.6 cm in diameter, the mice received the ¹⁷⁷Lu-labeled Hereceptin™ HERCEPTIN. The mice were injected with each RIC (about 5 µCi of each) intravenously (i.v.) *via* the tail vein. Mice (n = 5) were sacrificed by exsanguination at 24, 48, 72, 96 and 168 h.

Please replace the title of Table 2 with the following:

Table 2. Biodistribution of Hereceptin™ HERCEPTIN radiolabeled with ¹⁷⁷Lu using bifunctional chelates after intravenous injection: Percent Injected dose/gram

Please replace paragraph [0131] with the following:

[0131] The CHX-DOTA (14) RIC resulted in the highest values throughout the study. At 24 h, the femur %ID/g is 3.52 ± 0.67 , which then peaks at 96 h at 4.04 ± 0.88 . In contrast, the RIC consisting of the PA-DOTA ligand yielded the lowest femur %ID/g with 1.98 ± 0.17 , which declined to 0.97 ± 0.17 and 1.04 ± 0.16 at 96 and 168 h, respectively. The ^{177}Lu -C-DOTA and 1B4M-DOTA (7) ~~Hereceptin~~TM HERCEPTIN conjugates were not appreciably different from each other, and both were intermediate in the femur %ID/g as compared to the CHX- and PA-DOTA RIC. Differences among the ^{177}Lu -labeled RIC were also evident in the %ID/g calculated for the tumor xenografts and the other normal tissues that were collected (Table 2).

Please replace paragraph [0132] with the following:

[0132] The greatest uptake in tumor by a RIC was observed with the ^{177}Lu -1B4M-DOTA (7) ~~Hereceptin~~ HERCEPTIN. At 48 and 72 h the tumor %ID/g was 42.46 ± 12.35 and 39.22 ± 6.50 , respectively. The lowest values, 26.78 ± 3.25 and 24.23 ± 11.96 , were obtained with the CHX-DOTA (14) RIC at the same time points.

Please replace paragraph [0134] with the following:

[0134] In other normal tissues, the C-DOTA RIC resulted in the highest kidney %ID/g at each of the study time points with the exception of 168 h, at which a value of 2.79 ± 0.37 was obtained with the CHX-DOTA (14) RIC. The spleen %ID/g was the greatest with the PA-DOTA RIC from 24-72 h, while higher values were determined at 96 and 168 h with the C-DOTA RIC. The lowest spleen %ID/g values were obtained with ~~^{177}Lu -1B4M-DOTA-Hereceptin~~ ^{177}Lu -1B4M-DOTA-HERCEPTIN at 24, 48 and 168 h and with the CHX-DOTA (14) RIC at 72 and 96 h.

Please replace the footnote for Table 3 with the following:

^aAthymic mice bearing s.c.human colon carcinoma (LS-174T) xenografts were co-injected i.v. with approximately 2-5 μCi of ^{177}Lu -labeled immunoconjugates and ~~^{125}I -Hereceptin~~ ^{125}I -HERCEPTIN. The mice (n=5) were sacrificed by exsanguinations as described above. The blood, tumor and major organs were collected and wet-weighed, and the radioactivity was measured. The tissue-to-blood ratios were calculated for each tissue.